The presence of PD-L1 in men with localized prostate cancer

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Abstract

Background

Recent therapeutic strategies for different cancer types have focused on targeting immune check-points, such as programmed death-1 (PD-1) and its ligand PD-L1. However, it was recently reported that men with castration-resistant prostate cancer did not respond to PD-1 blockade as monotherapy. The unresponsiveness could potentially be explained by low expression of PD-L1 on prostate tumor cells. This study investigated the expression of PD-L1 on tumor cells and tumor infiltrating lymphocytes (TILs) in men with primary prostate cancer.

Material and Methods

Immunohistochemical analysis of PD-L1 expression was performed in a cohort of men undergoing transurethral resection of the prostate and diagnosed with prostate cancer. The expression was evaluated in tissue microarrays from 522 patients with at least 25 years of follow-up.

Results

Only four of the 522 evaluated cases were positive for PD-L1, positivity on tumor cells were found in three of the cases, of which one case also had positivity on TILs, while a fourth case only had positivity on TILs.

Conclusion

Our data suggest that treatments targeting the PD-1/PD-L1 interaction may not be successful as monotherapy in patients diagnosed with localized prostate cancer due to low expression of PD-L1.

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1. Background

1.1. Prostate cancer

Prostate cancer is the most frequently diagnosed malignant neoplasm among men, accounting for approximately 15% of all newly diagnosed male cancers worldwide (Ferlay et al. 2015). Patients with early-stage, localized prostate cancer are usually offered treatments with curative intention such as surgery or radiotherapy, alternatively active surveillance. For patients with metastatic prostate cancer, androgen deprivation therapy still is the standard treatment.

1.2. Cancer immunoediting

Decades ago, Burnet *et al.* postulated the ability of the immune system to detect and eliminate tumor cells and thereby play an important role in tumor growth inhibition. Since then, several studies have provided evidence that predominantly CD8 cytotoxic T cells is capable of recognizing and destroying various tumor cells (Appay et al. 2002, Carlos 2001, Nguyen et al. 1999). Thus, a consequence for patients in which the immune system has lost the capacity to detect and destroy transformed cells could be tumor development. The ability of tumor cells to escape the anti-tumor immune response, termed cancer immunoediting, is now considered as one of the hallmarks of cancer. The concept of cancer immunoediting includes three different phases; 1) the elimination phase, in which transformed cells are eradicated by an active immune response, 2) the equilibrium phase, in which some tumor cells survives and 3) the escape phase, in which the tumor cells progress in spite of an active immune response (Dunn et al. 2002).

1.3. Immune checkpoint regulator PD-L1

Cancer cells achieve to elude immunosurveillance through several immunosuppressive mechanisms, including the use of immune check points. In normal conditions, immune checkpoints are receptors expressed on T cells, which prevent excessive immune response against non-self antigens. The main immune checkpoints are the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the programmed cell death protein 1 (PD-1) and its ligand (PD-L1). CTLA-4 is expressed solely on T-cells while PD-1 is also expressed on B cells and natural killer (NK) cells. PD-1 is an inhibitory receptor, which when interacting with its ligand PD-L1 has the function to limit T cell effector functions and promote the differentiation of CD4 helper T cells into regulatory T cells (T_{regs}) with known immune suppressive function. One mechanism for tumor cells to avoid the anti-tumor immune response would be to over-express PD-L1 themselves, or to stimulate the PD-L1 expression on immune cells found within the tumor microenvironment.

The expression of PD-L1 has been investigated in several different cancer types, such as melanoma, renal-cell cancer, breast cancer and non-small-cell lung cancer (Brahmer et al. 2012, Katsuya et al. 2015, Nakanishi et al. 2007). Inhibition of the PD-1/PD-L1 interaction has recently proposed to increase antitumor immune response and represents the major advancement in cancer therapy in recent years. Monoclonal antibodies targeting immune check-points like ililimumab and tremelimumab (anti- CTLA-4), pembrolizumab and nivolumab against PD-1 and atezolizumab, avelimab and durvalumab (anti PD-L1) proved to be effective in clinical trials in melanoma, lung cancer and also in genitourinary malignancies (kidney and bladder cancer). Immune check-point inhibitors produce generally late but durable responses and in lung cancer response rates are associated with increased immunohistochemical expression of the PD-L1 protein (Brahmer et al. 2012, Massari et al. 2016, Topalian et al. 2012).

Unfortunately, the effect of PD-1/PD-L1 blockade has not been proven successful in prostate cancer so far. It has been speculated that low expression of PD-L1 on prostate cancer cells might account for the lack of objective response. The few available reports on the expression of PD-L1 on prostate tumor cells have provided conflicting results (Gevensleben et al. 2016, Martin et al. 2015, Massari et al. 2016, Taube et al. 2014, Topalian et al. 2012). Topalian *et al.* reported

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low levels of PD-L1 expression in prostate cancer tissue obtained from men with castration-resistant prostate cancer. Similarly, scarce PD-L1 expression was shown by Martin *et al.* investigating 20 primary prostate cancer specimens (Martin et al. 2015, Topalian et al. 2012). On the other hand, Massari *et al.* reported high levels of PD-L1 in 19% of the 16 men with castration-resistant prostate cancer investigated (Massari et al. 2016). In line with the results by Massari, Gevensleben *et al.* detected moderate to high PD-L1 expression in 62% of their cohort comprising 640 men having undergone radical prostatectomy (Gevensleben et al. 2016). Unfortunately, different antibodies for the IHC detection of PD-L1 have been utilized in these reports. The use of incomparable reagents may have introduced a significant bias in the immunohistochemical detection of PD-L1 across different laboratories. In addition, none of the previous studies has investigated the potential prognostic role of PD-L1 in well annotated cohorts of prostate cancer patients.

In this study, the level of immunohistochemical expression of PD-L1 on tumor cells and tumor infiltrating lymphocytes (TILs) was evaluated in a cohort of men diagnosed with localized prostate cancer and followed up to 30 years.

2. Material and methods

2.1. Case and tissue selection

The present study is nested within a cohort of men diagnosed with localized prostate cancer diagnosed in the Örebro and South East Health Care Region of Sweden between 1977 and 1999, as described previously (Andren et al. 2006, Johansson et al. 2004). Initially, a cohort of 1367 men was identified during the study period. Eligible patients were identified through the population-based prostate cancer quality database in these regions. Men that were diagnosed with incidental prostate cancer through transurethral resection of the prostate (TURP), i.e. category T1a-b tumors was included in the cohort. In accordance with standard treatment protocols at that time, patients with early localized prostate cancer were followed expectantly.

The study cohort was followed for cancer-specific and all-cause mortality until 1 March 2006, through record linkages to the Swedish Death Register and Migration Register. Information on cause of death was obtained for each individual through a complete review of medical records by a study end point committee. Deaths were classified as cancer specific when prostate cancer was the primary cause of death. In this study, a nested study design was used, that included men who either died from prostate cancer during follow-up or who survived at least ten years following their diagnosis. The study design excluded men with non-informative outcomes who either died from other causes within 10 years after cancer diagnosis, or did not die of prostate cancer and did not have the opportunity to survive years before the end of study follow-up. Cases without complete immunohistochemistry data were also excluded.

2.2. Immunohistochemistry

Tissue cores with a diameter of 0.6 mm were collected from the transurethral resection of the prostate specimens belonging to each patient. To address potential tumor heterogeneity, three cores were taken from each patient. Tissue microarrays (TMAs) were constructed with a Beecher manual arrayer and 4 µm tissue microarray sections from the TMA blocks were used for IHC. Immunohistochemical analysis for PD-L1 was tested first on whole sections of five selected recent specimens to reach optimal conditions on the automated Benchmark; Ventana ULTRA Staining platform. Optimized conditions included slide steam heating at 100 C with EDTA for 92 minutes in pH 8; 1 hour incubation with the primary rabbit monoclonal antibody anti-PDL1/CD274 (clone SP142;Spring) diluted 1:30; and revelation with the amplification system U OptiView DAB IHC v5.

2.3. Evaluation of PD-L1 expression

The immunostaining was assessed by two observers (SD and JC) blinded to all the clinical data and independently as follows. Each core was semi-quantitatively deemed to be populated by at least 1000 neoplastic cells. Specific membranous and/or cytoplasmatic staining of epithelial tumor cells and TILs was

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considered as positive. Accordingly, the percentage of PD-L1 staining in tumor cells and TILs in prostate cancer cores were evaluated separately. The immunostaining was scored as 1+ when <5% positive cells were counted; 2+ when the percentage of stained cells was >5% to <50%; and 3+ when the number of stained cells was >50%.

3. Results

In this study, the immunohistochemical expression of PD-L1 was evaluated on TMA blocks from the Swedish Watchful Waiting Cohort. Of the 735 original cases included on the TMAs, 213 cases were excluded due to lack of enough tissue material for evaluation; leaving 522 cases which could be evaluated for immunohistochemical PD-L1 expression. The clinical characteristics of the cases are presented in Table 1.

Only four of the 522 evaluated cases were found to be positive for PD-L1. Positivity on tumor cells was found in three of the cases, where PD-L1 expression was found as membranous or cytoplasmic staining (Figure 1). Furthermore, positive membranous and cytoplasmic staining was found on TILs in two cases. All cases showed a 1+ immunoreactivity, i.e. <5% of positive cells (Table 2). Further investigations of the positive cases revealed that the three cases with positivity on tumor cells all died from prostate cancer, while the one case with only positivity on TILs died from other causes than prostate cancer.

4. Discussion

Increased expression of PD-L1 on tumor cells and TILs have previously been reported for a number of different malignancies, including melanoma, pancreas, lung, kidney, and cervical cancer (Karim et al. 2009, Konishi et al. 2004, Nakanishi et al. 2007, Taube et al. 2014). Recently it has been suggested that immunohistochemically identified PD-L1 is a potential marker for a successful treatment directed at the PD-1/PD-L1 interaction. However, inhibitors against PD-1/PD-L1 have not yet shown satisfactory outcomes in prostate cancer and low tumor PD-L1 expression has been suggested as a potential reason for this lack of objective response. Our investigation revealed scarce PD-L1 expression in prostatic tissue obtained from men diagnosed with incidental prostate cancer through TURP. All of the 522 cases included in this study showed no or low (<5%) immunohistochemical staining of PD-L1 on prostate cancer cells and TILs. Our data indicates that treatments directed at the PD-1/PD-L1 interaction are unlikely to be successful as therapy for men diagnosed with primary prostate cancer due to this low expression of PD-L1.

A multicenter phase I trial recently assessed the safety and activity profile of nivolumab in a cohort of 296 patients with advanced solid tumors, including 17 castration-resistant prostate cancer (CRPC) patients (Topalian et al. 2012). The anti-PD-1 treatment produced objective response in patients diagnosed with melanoma, non-small-cell lung cancer, and renal cancer. Unfortunately, no objective response was detected in men with CRPC. Further notably in Topalians study was that no PD-L1 expression was reported for the two CRPC specimens eligible for immunohistochemical analysis. Even though the number of evaluated patients was very low, the results are in line with our findings. Additional evidence for low PD-L1 expression on prostate cancer cells was reported in a study performed by Taube *et al.*, investigating PD-L1 expression in 41 patients with advanced, treatment-refractory tumors including melanoma, renal cancer, lung cancer, CRPC, and colorectal cancer. The results revealed significantly varied PD-L1 expression between different tumor types, most abundant in melanoma, renal cancer, and lung cancer in contrast to CRPC and colorectal cancer where the expression was scarce (Taube et al. 2014). To our knowledge, only one study has used whole–mount prostate cancer sections for immunohistochemical evaluation of PD-L1 expression on tumor cells (Martin et al. 2015). In this study, IHC for PD-L1 was performed on 20 whole-mount sections containing primary prostate cancer specimens and the presented results were similar as in the present study, i.e. PD-L1 expression was scarce in primary prostate tumors. In summary, these reports support the theory that a potential reason to the disappointing effects of immune

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checkpoint blockade (anti-PD-1 and anti-PD-L1) in prostate cancer is that prostate tumors have no or limited PD-L1 expression.

However, conflicting results have been reported, showing high PD-L1expression in both primary prostate cancer and CRPC specimens. In a study by Massari *et al*. involving 16 CRPC specimens, 56% of the cases expressed PD-L1, among them 19% expressed high levels (Massari et al. 2016). As similar percentage of positivity was detected in a comprehensive study performed on two independent cohorts of primary prostate cancer patients (Gevensleben et al. 2016). The authors in these two studies concluded that PD-1/PD-L1-targeted therapy may be an option for prostate cancer patients with primary prostate cancer or CRPC.

The disparity between the studies could be due to several possible explanations. The first explanation could be the use of incomparable reagents and the fact that there is no standard method for quantifying PD-L1 expression with IHC. Second, studies have reported focal PD-L1 expression which can potentially be missed in small tumor specimens, ending up in a false-negative PD-L1 evaluation (Kitazono et al. 2015). As TMAs were utilized in the present study, the PD-L1 expression may have been under-estimated due to small tumor samples. A second reason for an under-estimation of the PD-L1 score could be the possibility that the immunoreactivity for PD-L1 fades over time. The tissues used in the present study are relatively old, which can be viewed as a study limitation.

Two different mechanisms have been suggested for triggering PD-L1 expression on tumor cells, where the most common is driven by pro-inflammatory cytokines, such as INF- γ, produced during an active anti-tumor immune response (Taube et al. 2012, Tumeh et al. 2014). This process, termed adaptive immune resistance, will increase the PD-L1 expression on tumors developing in a microenvironment consisting of CD8 cytotoxic T cells. If a locally immunosuppressive environment exists in the tumor cores evaluated in this study, it may also have an influence on our results since it could contribute to decreased PD-L1 expression. We have previously shown substantial infiltration of T_{regs} in this material which indicates an immunosuppressive micro milieu (Davidsson et al. 2013).

To further elucidate the potential of PD-1/PD-L1-targeted therapy in prostate cancer, future studies are needed to clarify if PD-L1 expression can be used as a marker to identify patients that will benefit from the treatment.

5. Conclusion

Our data suggest that treatments targeting the PD-1/PD-L1 interaction may not be successful as monotherapy in patients diagnosed with primary prostate cancer.

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References

1. Andren O, Fall K, Franzen L, et al. How well does the Gleason score predict prostate cancer death? A 20-year followup of a population based cohort in Sweden. In: J Urol. 2006/03/07 ed.: 2006. p. 1337-40.

2. Appay V, Zaunders JJ, Papagno L, et al. Characterization of CD4(+) CTLs ex vivo. In: J Immunol. 2002/05/23 ed.: 2002. p. 5954-8.

3. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. In: N Engl J Med. ed.: 2012. p. 2455-65.

4. Carlos TM. Leukocyte recruitment at sites of tumor: dissonant orchestration. In: J Leukoc Biol. 2001/08/09 ed.: 2001. p. 171-84.

5. Davidsson S, Ohlson AL, Andersson SO, et al. CD4 helper T cells, CD8 cytotoxic T cells, and FOXP3(+) regulatory T cells with respect to lethal prostate cancer. In: Mod Pathol. 2012/10/09 ed.: 2013. p. 448-55.

6. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. In: Nature immunology. ed.: 2002. p. 991-8.

7. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. In: Int J Cancer. ed.: 2015. p. E359-86.

8. Gevensleben H, Dietrich D, Golletz C, et al. The Immune Checkpoint Regulator PD-L1 Is Highly Expressed in Aggressive Primary Prostate Cancer. In: Clin Cancer Res. ed.: 2016. p. 1969-77.

9. Johansson JE, Andren O, Andersson SO, et al. Natural history of early, localized prostate cancer. In: JAMA. 2004/06/10 ed.: 2004. p. 2713-9.

10. Karim R, Jordanova ES, Piersma SJ, et al. Tumor-expressed B7-H1 and B7-DC in relation to PD-1+ T-cell infiltration and survival of patients with cervical carcinoma. In: Clin Cancer Res. ed.: 2009. p. 6341-7.

11. Katsuya Y, Fujita Y, Horinouchi H, et al. Immunohistochemical status of PD-L1 in thymoma and thymic carcinoma. In: Lung Cancer. ed.: 2015. p. 154-9.

12. Kitazono S, Fujiwara Y, Tsuta K, et al. Reliability of Small Biopsy Samples Compared With Resected Specimens for the Determination of Programmed Death-Ligand 1 Expression in Non--Small-Cell Lung Cancer. In: Clinical lung cancer. ed.: 2015. p. 385-90.

13. Konishi J, Yamazaki K, Azuma M, et al. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. In: Clin Cancer Res. ed.: 2004. p. 5094-100.

14. Martin AM, Nirschl TR, Nirschl CJ, et al. Paucity of PD-L1 expression in prostate cancer: innate and adaptive immune resistance. In: Prostate cancer and prostatic diseases. ed.: 2015. p. 325-32.

15. Massari F, Ciccarese C, Calio A, et al. Magnitude of PD-1, PD-L1 and T Lymphocyte Expression on Tissue from Castration-Resistant Prostate

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Adenocarcinoma: An Exploratory Analysis. In: Targeted oncology. ed.: 2016. p. 345-51.

16. Nakanishi J, Wada Y, Matsumoto K, et al. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. In: Cancer Immunol Immunother. ed.: 2007. p. 1173-82.

17. Nguyen T, Naziruddin B, Dintzis S, et al. Recognition of breast cancer-associated peptides by tumor-reactive, HLA-class I restricted allogeneic cytotoxic T lymphocytes. In: Int J Cancer. 1999/05/04 ed.: 1999. p. 607-15.

18. Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. In: Science translational medicine. ed.: 2012. p. 127ra37.

19. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. In: Clin Cancer Res. ed.: 2014. p. 5064-74.

20. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. In: N Engl J Med. ed.: 2012. p. 2443-54.

21. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. In: Nature. ed.: 2014. p. 568-71.

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Table 1. Clinical and histopathological characteristics in the Swedish Watchful Waiting Cohort (N =522).

	N=522
Age at Dx, Mean (range)	73 (51-91)
Calendar year of Dx	
1977 – 1981	17 (3.3)
1982 - 1986	28 (5.4)
1987 – 1991	218 (41.8)
1992 – 1999	259 (49.6)
Tumor stage	
T1a	205 (39.3)
T1b	317 (60.7)
Gleason Score	
2-6	225 (43.1)
3+4	103 (19.7)
4+3	83 (15.9)
8-10	111 (21.3)
Tumor percent (52 missing)	
Q1: ≤2	157 (30.1)
Q2: >2 - ≤10	124 (23.8)
Q3: >10 - ≤25	80 (15.3)
Q4: >25	109 (20.9)

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Table 2. Characteristics of PD-L1 positive cases.

Case no.	*Tumor/TILs*	*Staining location*	*% positive cells*	*Gleason score*	*T-stage*	*% cancer*	*Lethality*
1	Tumor	Cytoplasmic	< 5	10	T1b	5	PCa
2	TILs	Membranous	< 5	6	T1b	15	Other
3	Tumor	Membranous	< 5	6	T1a	5	PCa
4	Tumor + TILs	Membranous + cytoplasmic	< 5	9	T1b	90	PCa

TILs – Tumor infiltrating lymphocytes

PCa – Prostate cancer

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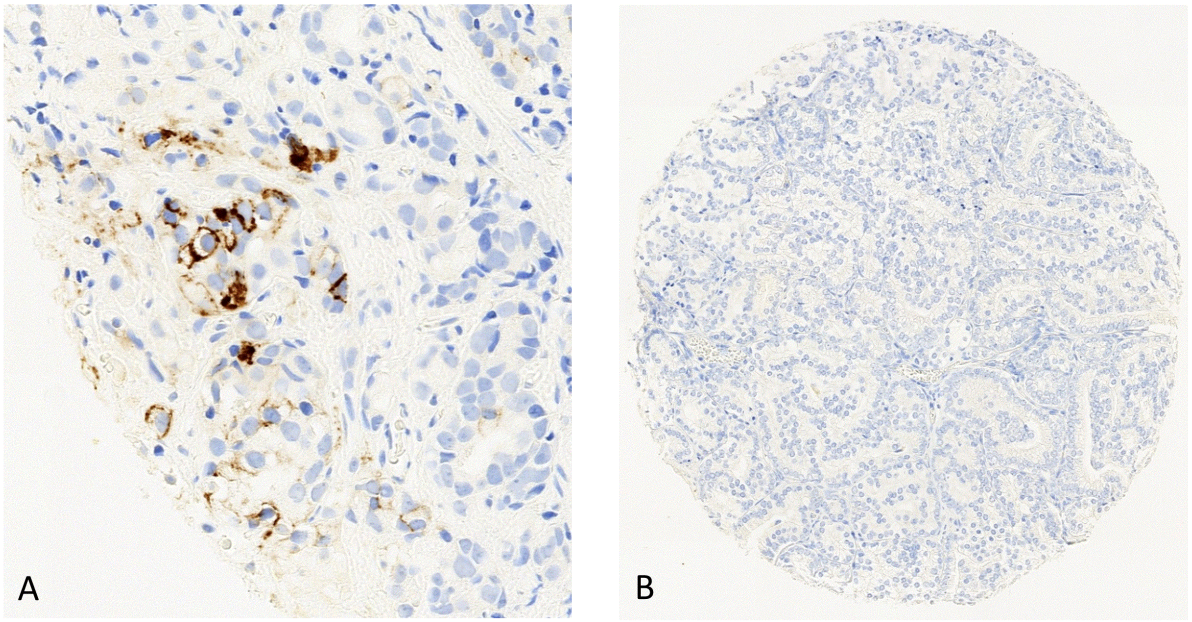

Figure 1. Immunostaining patterns of PD-L1 with the clone SP142 in prostate tissue. A) Positive membranous staining of tumor cells at 40X magnification, B) Negative staining at 20X magnification.

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